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  We file an application under Article 42 of Patent Act, file a Request for Examination under Article 60 of the same Act. Agent Sang-Hun Heo (Signature)

- Official Fee

Application Fee	20 page(s) 29,000 won.
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- Attached Documents

  1. The document 1 container which proves to correspond to medium and small firm by summary · specification (drawing) 1 container 2 medium and small firm. law enforcement ordinance article 2.

Patent Specification

Abstract

The present invention relates to the instillation gel agent using the surface of eyeball disease for treating amniotic extract and a method of manufacture thereof and such amniotic extract, and the eye drop or the instillation Ointments.

- Representative Drawing

  Drawing 3

Index Term

The healing ocular surface diseases, amniotic extract, instillation gel agent, eye drop, the instillation Ointments.

The Title of Invention

Amnionic extract for ophthalmopathy{Amnionic extract for ophthalmopathy}

Brief Discription of the Drawings

Fig. 1 compares the pre-ejection period result at the extracted amnion component with the amnion of the extraction former.

Figure 2 is a graph showing each group liver change of the epithelium defect area.

Figure 3 is a photograph showing the healing effect at the wound healing. A, B, and C relate to D the control group, E, and F is the experimental group. 3 ***, B , and E 2 weeks, C , and F are the photo filmed on 5 weeks.

Figure 4 is a graph showing each group liver change of the calcitonin by the corneal edema.

Figure 5 is a photograph showing the difference of the cornea cloudiness. A1, and A2 relate to B1 the control group, and B2 is the experimental group.

The Detailed Description of Invention

The Purpose of Invention

Field of Invention and the Prior Art

The present invention relates to the instillation gel agent using the surface of eyeball disease for treating amniotic extract and a method of manufacture thereof and such amniotic extract, and the eye drop or the instillation Ointments.

The incurability inflammation of the cornea represented as the surface of eyeball disease is the serious disease which in which the chronic inflammation, a hazy and new vessels etc. are generated in the cornea surface and causing the real name and shows a rejection after the corneal transplant. It tells to the blurred vision or the real name due to the damage of the corneal tissue it is possible to the perfect cure if the proper treatment is made but serious if a treatment is delayed at its early stage. The incurability inflammation of the cornea is caused by the pathogen infection, the chemical substance and immune reaction etc. Excessive the fact that the proteinase of a host and pathogen importantly acts is clarified in this progressing and pathogenesis [Fini ME, Girard MT, Matsubara M. Collagenolytic/Gelatinolytic enzymes in corneal wound healing. Acta Ophthalmologica. vol 70. 1992], it uses an antibiotic and the method at the same time. for administering the proteinase inhibitory substance. But the method does not obtain the big effect of remedy [Stuart JC, Turgeon P, Kowalski RP. Use of aprotinin in the treatment of pseudomonas corneal ulceration. Trans Pa Acad Ophthalmol Otolaryngol. 41. 1989: Byon DS, Kim JC, Shyn KH. The effect of synthetic inhibitor of metalloproteinase on corneal alkali burn in rabbit. Chung-Ang J Medicine. 20(3), 1995: Wentworth JS, Paterson CA, and Gray RD. Effect of a metalloproteinase inhibitor on established corneal ulcers after an alkali burn. Invest Ophthalmol Vis Sci. 33, 1992].

Up to date, in the healing ocular surface diseases including the incurability inflammation of the cornea, the special method is not developed the special method does not have the special method besides the method for administering an antibiotic to the damaged cornea in order to prevent the infection of a pathogen from.

Therefore, the surface of eyeball disease development of treatment including the incurability inflammation of the cornea is desperately required.

Technical Problems to be solved by the Invention

This inventor knew to could obtain the remarkable inflammatory control effect if it transplanted an amnion to the cornea surface through the extremely damaged empirical incurability inflammation of the cornea animal experiment after the superficial layer kerectomy. As a result of performing the thereafter many clinical study, this inventor confirmed that it had an effect in the various surface of eyeball disease. And it could obtain the good result from the temporary amnion grafting application from the incurability inflammation of the cornea model using the animal of the alkali model than the control group.

Thus, it had the assurance that the material contributing to the healing ocular surface diseases including the inflammation of the cornea was included within the amnion component. An amnion was transplanted from all kinds of the incurabilities the inflammation of the cornea and surface of eyeball disease and an effect could be actually obtained from a clinical in the treatment of the inflammation of the cornea. Besides, recently, the excellent effect could be obtained, it knew at the prevention of the corneal opacity most indicated into a problem in the vision with correction laser operation very much carried out.

The present invention obtained the amniotic extract of composition with a superior activity the healing effect is in the cell damage suppression by a proteinase and incurability inflammation of the cornea.

Therefore, it is an object of the present invention to provide the eye drops (the gel type or the water soluble ophthalmic) for the healing ocular surface diseases which is manufactured by using the amniotic extract or the instillation Ointments.

The Structure and Function of the Invention(Device)

The present invention has the effective amniotic extract as the feature in the healing ocular surface diseases.

Moreover, the present invention is a measure. The amniotic extract is done by the effective component it decides on the eye drops or the instillation Ointments in which the normal antibiotic is together contained to and, the other feature if necessary.

Moreover, the present invention has the manufacturing method of the amniotic extract in which the process of filtering by using the extrinsic filtration membrane filter (Centrikon) doing the supernatant adding the liquid nitrogen in the process: c) amnion stock solution of dipping an amnion in the stock solution in which the process: b) DMEM (Dulbecco's Modified Eagle's Medium) cell culture medium of peeling off an amnion from the a) placenta, and the glycerol and DMSO (dimethyl sulfoxide) are contained and homogenizes the process: d) crushed material of pulverizing by using the mortar of the freeze-drying state through an equalizer and performs a centrifuge and is extracted with the process and e) above statement of obtaining a supernatant is included as and, the other feature.

If described in more detail, it is this kind of present invention the same like a next.

The present invention relates to the increased amniotic extract which can be widely applied to as all kinds of the surface of eyeballs the therapeutic agents and aiding agent.

As described in detail, it is the amnion extracting procedure the same to the by processes like a next.

In the placenta of the woman delivered of a child, an amnion is desquamated. After the amnion peeled off is washed to the saline solution in which an antibiotic is mixed, it keeps in the stock solution in which the DMEM cell culture medium, and the glycerol and DMSO are contained with a soak. The liquid nitrogen is added in the stock solution in which an amnion is included and it is frozen and dried. By using the mortar of the state dried in freezing, the state pulverizes. As to the crushed material, after homogenizing by using an equalizer, it does with centrifuges (3000 ~ 6000 rpm, 0.5 ~ 1 hour) and it is a supernatant attended by the drunken fellow. By filtering by using the extrinsic filtration membrane filter (Centrikon) a supernatant the present invention obtains the intended the amniotic extract. At this time, process of drying the filtered amniotic extract and pulverizing can be added.

The amniotic extract obtained by the sampling method described in the above is and saves deposited, the methyl cellulose of 1 : 0.5 ~ 2 volume ratio is mixed with the amniotic extract and it puts into the disease in which an anti-ultraviolet is possible and it refrigerates and keeps at low temperature. Moreover, when applying to a patient as the surface of eyeball therapeutic agents, an antibiotic, the hyalouronic acid (Hyaloronic acid) etc. are mixed according to a need to 0.01 ~ 99.9 weight% range and a patient can use.

In the meantime, the present invention is to be equipped with the eye drops which it has the above-described amniotic extract as the effective component, it does. The base or the excipient used with the eye drops (the gel form, the aqueous solution type etc) or the instillation Ointments in manufacture is the normal thing used in the ophthalmology field. Moreover, the eye drops which the amniotic extract is to the effective component, it does drops lotion with 1 1 ~ 5 time. And 1 eye drop amount is 1 ~ 500 mg (the effective ingredient content) range. But the instillation times and eye drop amount can be changed according to the state of a patient.

As illustrated in the above, it contains in the adjuvant treatment: dry eye for maximizing the effect of the amnion grafting which the amniotic extract uses parallel with deficit part treatment generated around the therapeutic agent: in the complication generation after the fast curative: excimer Rasik operation of the corneal opacity suppression after the therapeutic agent: excimer Rasik operation of the eye drops having with measure it is done by the effective component or the persistence epithelium defect which the instillation Ointments includes the therapeutic agent of all kinds of the incurabilities the surface of eyeball disease, and the canker of a cornea including eyeball image, stevens-Johnson C syndrome, and herpes inflammation of the cornea and epithelial cell and cornea surgery, the lesion removal: proposal and Reconstruction, including, the stabilizer: antibiotic of the surface of eyeball inflammation by the use which becomes wrong of the aiding agent: contact lenses at the moltinism type field or the long term usage, including, 4-diaminodiphenyl sulfone: hyalouronic acid or the contact lens etc., is carried out on the effect of medical treatment enlargement: existing clinical in the artilicial tear and it can use.

This kind of present invention more circumstantially will illustrate based on the following embodiment. And the present invention is not thus restricted.

According to the research result of this inventor, it could find out that the A1- antitrypsin, inter -a1- antitrypsin, anti -a2- macroglobulin, anti -a2- antichymotrypsin, anti -a2- anti-plasmin suppressing the serine protease existed among the proteinase which the pathogenic microorganism inducing the inflammation of the cornea in the amnion which collected with an aseptic secreted. Moreover, in the TNF (tumor necrosis factor) -a and the amnion handled with the LPS (lipopolysaccharide), whereas the MMP (matrix metalloproteinase) -2, 9 and TIMP (tissue inhibitor of metalloproteinases) -1, and the mRNA expression of 2 were increased. MMP-2 , and the expression of 9 were not observed in the processing proamnion. And TIMP-1, and the mRNA of 2 and protein expression were observed. In the hydrogen peroxide, and a post-administration the amnion milling liquid than the xanthene / xanthine oxidase and nitric oxide single injection time, the DNA damage was more less and the high cell viability was shown. The silencing of the iNOS (inducible nitric oxide synthase) mRNA was observed while it lost the NO (nitric oxide) amount of the culture fluid. The caspase - 3 (Caspase-3) activity which was increased after administering TNF-a reduced the amnion milling liquid in an administration. Moreover, in an amnion, the heat shock protein 27, 47, 70, 90 expression was observed. And it was confirmed [refer to Figure 1] to the peculiar matter at the extraction proamnion be concentrated and detected on the pre-ejection period inspection about the amnion extract

The amnion transplant is known that the amnion transplant clinically has an inflammation and cell apoptosis deterrent effect. And a related has many genes with this. Moreover, TNF, and IL-1 increase and it is known to the tear or the front in an inflammation. Thus, when this inventor together added the extract of an amnion and the time which the corneagen cell added to vitro only the inflammatory cytokine and it did the cultivation, this inventor confirmed as a method as follows whether the expression of the apoptosis gene and inflammation could be held back.

After an amnion was peeled off from a placenta, it washed to the saline solution in which an antibiotic was mixed. The soak *** in the stock solution in which the DMEM cell culture medium, the glycerol, and DMSO are contained. The liquid nitrogen was added in the stock solution in which an amnion was included and it was frozen and dried. By using the mortar of the state dried in freezing, the state pulverized. As to the crushed material, after homogenizing by using the equalizer (homogenizer), it did with centrifuges (5000 rpm, 1 hour) and it was a supernatant a withdrawal. After using 0.22 microfilter, the extracted supernatant was filtered, it partitioned over 100 kDa less than 100 ~ 30 kDa, 30 kDa, 10 kDa, 10 kDa by using the centrifugal ultrafiltration paper. All processes were made in 4 ℃. And the determination of protein and the fractionated amnion component determined the amount of administration. It divided into the group which independently,singly added TNF and IL-1 in the human corneal fibroblast and the group which together added the extract of an amnion and the group did the cultivation.

And in the cell survival measurement (MTT assay), the morphology of a cell, the discriminative gene expression (differential gene expression) observation using the cDNA analysis (array), and the culture medium, the Western blot and zymography were implemented on a measurement, and the culture medium and cell lysate and a confirmation , and the caspase -3 activity measured an expression and protease activity of MMP the NOx generation rate at HPLC. The mRNA expression of MMP and iNOS was measured through the RT- PCR (Reverse Transcriptase PCR) trial.

Consequently, in the amnion component dosage group, the survival rate about a cell was high. In the culture medium of the amnion component dosage group, the NO generation rate was reduced. An iNOS an expression was reduced in RT-PCR and cDNA analysis. The caspase -3 activity reduced according to the amount of administration when injecting the amnion component. The caspase -3 activity reduced with gene expression diagram. In the lower layer solution than a supernatant, MMP-1, and 2 were very much measured. And an expression was supressed in the amnion component post-administration cultivation. In the RT-PCR about MMP-2, an expression was supressed at the amnion dosage group treated with TNF. But it was nearly similar in the amnion dosage group treated with IL-1. Moreover, the MMP-1, 2, 3, 9 was similar or it was a little bit increased on the cDNA analysis.

Therefore, the amnion component can know a holding the cytoprotective effect. Moreover, the amnion component suppressed the expression of the induction of inflammation gene. It prevented the cell apoptosis. And it seemed to accelerate the specialization of a cell. And through this, the amnion component suppressed the tissue injury. It could know to suppress the progress of the tissue injury.

As a result of implementing 3 days temporary amnion grafting about the edge discussion cornea plating the alkali burn, it could discover that the wound healing was rapidly made than the inflammation cell permeation protecting and metalloproteinase deterrent effect including the polymorphonuclear leukocyte etc. Therefore, it recognized due to the method as follows whether it could expect the same effect in case of pulverizing an amnion and not making the eye drop and using or not.

After after precipitating the circular filter paper of the diameter 6 mm in 1 N NaOH solution in the rabbit inside 28, this being applied to a cornea with 30 the first publication and removing, it washed to the saline solution to 5 cc. It classified into groups (the group 1, and a n=7), using only the amnion milling liquid the amnion milling liquid and groups (the group 2, and a n=7), using the methyl cellulose 1 : 1 mixed solution groups (the group 3, and a n=7) using as the control group only the methyl cellulose, and groups (the group 4, and a n=7) which nothing did not use as the second opinion control group. And a target was applied between the respective 1 week with the day 4 times sick. As to the Corneale defect area, after projecting 35 mm photography diapositive film and scanning to the video camera, by using the area measurement program on a monitor, it measured this. The change of the calcitonin was measured for the determination of the corneal edema in the cornea central part by using the pachymeter. The measurement of the identical with every week method was implemented to the experiment 5 weeks.

Consequently, as to the Corneale defect, the regeneration speed was fast but a difference between the experimental group a meaning altogether statistically did not have experimental group (the group 1, and the group 2) applying the amnion milling liquid in comparision with control group (the group 3, and the group 4) less than P value 0.05. In each group, while a difference passed the control group and the amnion milling liquid which did not apply the amnion milling liquid from 3 between the experimental group dropping lotion, thereafter the epithelium defect area after the alkali burn was continuously,consistently significantly a maintains to 5 weeks. In the experimental group in 2 ~ 3 weeks the recurrens epithalaxia of an epithelium happens, constanted, whereas the epithelium defect area was decreased, relatively the recurrens epithelium defect was shown up in the control group. Thereafter, in 5 weeks, the clear difference was shown between the control group and experimental group [fig. 2 a , refer to Figure 3].

As to the corneal edema, after the experimental group showed the phase in which the corneal edema was less than those of the control group during 1 week, thereafter it did not show the peculiar difference between the control group and the experimental group according to a time-out. But an edema was decreased in the final 5 weeks and an edema of the experimental group was less in comparision with the control group [fig. 4 a , refer to Figure 5] to statistically have with meaning.

The next table 1 showed the cloudiness of the cornea measured in 2 weeks and 5 weeks. The cloudiness of the experimental group was less in 2 weeks and 5 weeks in comparision with the control group.

The manufacturing method of the instillation gel agent containing the amniotic extract of the present invention is as follows.

Amniotic extract 5 ㎎.

Carbopol 934 20 ㎎.

Triethanolammine dosage.

Para auxin benzoic acid methyl 2 ㎎.

Sterile pure water ad. 1 g/6.

After the para auxin benzoic acid methyl being added to the sterile pure water and heating and dissolving, it cooled and the amniotic extract was dissolved. Here, after the Carbopol 934 being added and mixing to the high-speed agitator and dispersing, it assumed the helm of state and an atmosphere was terminated. Here, while it noted while adding a triethanolammine the drop by drop so that the air be necessary, it agitated and it manufactured

The manufacturing method of the eye drop containing the amniotic extract of the present invention is as follows.

Amniotic extract 5 g/6.

Benzalkonium chloride 0.1 g/6.

Sodium chloride 5 g/6.

Boric acid 6.2 g/6.

Tyloxapol 1.0 g/6.

The watery proper amount of hydrochloric acid.

Sterile pure water ad. 1000 ㎖.

In order, the amniotic extract, the sodium chloride, and the boric acid were injected in the amniotic extract and it dissolved. The benzalkonium chloride which here it dissolved in a small amount of sterile pure water, and a tyloxapol were added and it agitated. The dilute hydrochloric acid was added and a pH was controlled. The sterilization performed by using 0.45 microfilter.

The manufacturing method of the instillation Ointments containing the amniotic extract of the present invention is as follows.

Amniotic extract 5 ㎎.

Sterile pure water 10 ㎎.

Paraoxybenzoic acid methyl 2 ㎎.

Anhydrous lanolin 100 ㎎.

Petrolatum album ad. 1 g/6.

It was the amniotic extract and paraoxybenzoic acid methyl a withdrawal in the glass bowl sterilized. After the amniotic extract was added and it melted, here until while adding the anhydrous lanolin, it combined and it homogenized, it mixed and it manufactured.

The virulence test was performed about the positive number extract of the present invention like a next. Concretely, after administering the amniotic extract to female and male rabbit (5 head per the group) to the abdominal cavity and buccal cavity according to the respective concentration, during being 14, an extract was observed and a mortality was measured. LO the death example is not observed in the capacity which is the administration highest capacity of 5 ㎖ per the weight kg at all.
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As to the calculation of a value, it was impossible.

Effect of Invention(Device)

As illustrated in the above, as to the amniotic extract, since being effective in all kinds of the healing ocular surface diseaseses, it is formulated to the instillation therapeutic agent, for example, the instillation therapeutic agent, for example, the eye drop, the instillation gel agent, and the instillation Ointments and it conveniently can use.

Claim [1]

The amniotic extract, wherein it is effective in the healing ocular surface diseases.

Claim [2]

The eye drops, wherein it has with measure the amniotic extract is done by the effective component.

Claim [3]

The eye drops of claim 2, wherein it has with measure the antibiotic or the hyalouronic acid is done by 0.01 ~ 99.9 weight% about the amniotic extract.

Claim [4]

The eye drops of claim 2 or 3, wherein the eye drops is the gel form or the aqueous solution type.

Claim [5]

The instillation Ointments, wherein it has with measure the amniotic extract is done by the effective component.

Claim [6]

The instillation Ointments of claim 5, wherein it has with measure the antibiotic or the hyalouronic acid is done by 0.01 ~ 99.9 weight% about the amniotic extract.

Claim [7]

a) With the process, of dipping an amnion in the stock solution in which the process, of peeling off an amnion from a placenta the b) DMEM (Dulbecco's Modified Eagle's Medium) cell culture medium, and the glycerol and DMSO (dimethyl sulfoxide) are contained the process of pulverizing it uses the mortar of the freeze-drying state it adds the liquid nitrogen in the c) amnion stock solution, and the process of homogenizing the d) crushed material with equalizer and performing a centrifuge and obtaining a supernatant.

e) The manufacturing method of the amniotic extract wherein it is made of process of filtering by using the extrinsic filtration membrane filter (Centrikon) doing the extracted supernatant.

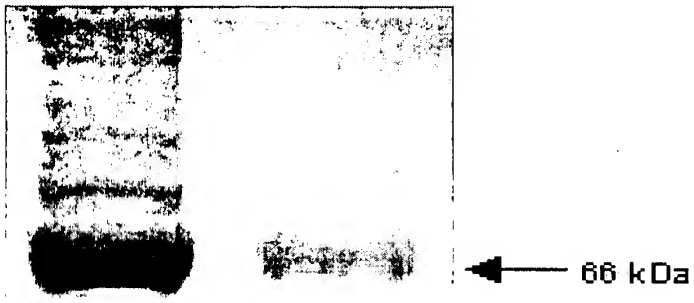
Claim [8]

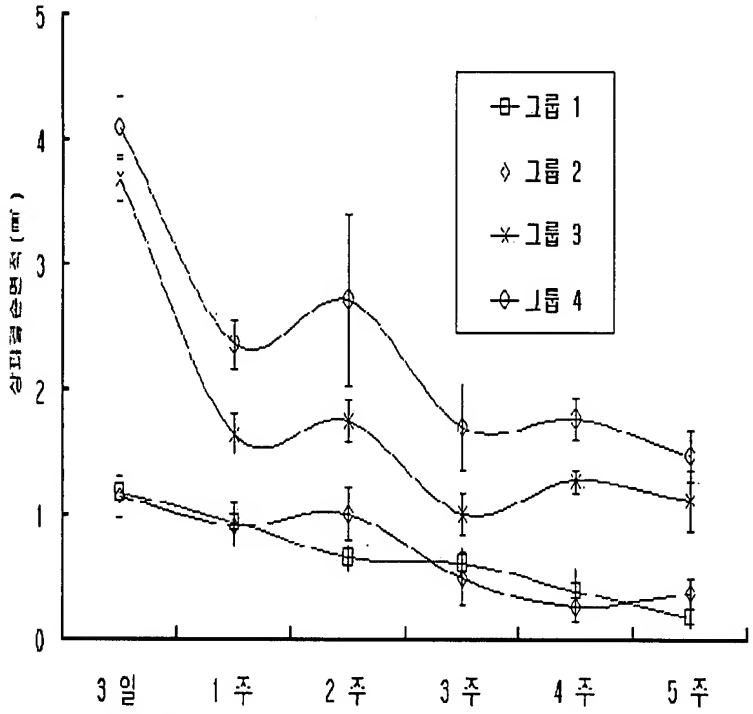
The manufacturing method of the amniotic extract of claim 7, wherein the process of drying the filtered amniotic extract as described above and pulverizing is the fall crop measure is a thing done by a feature.

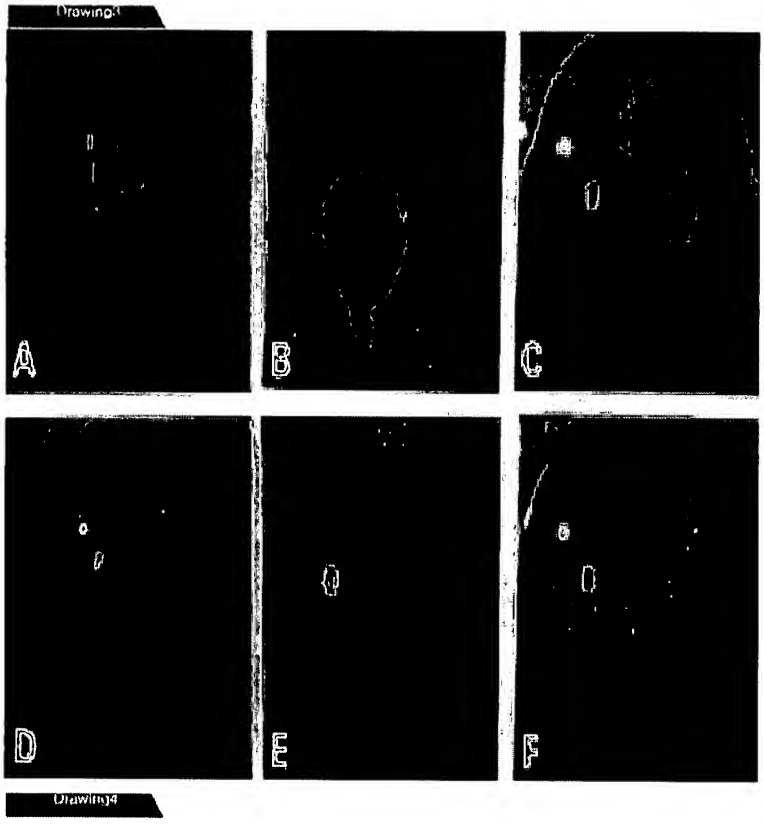
Claim [9]

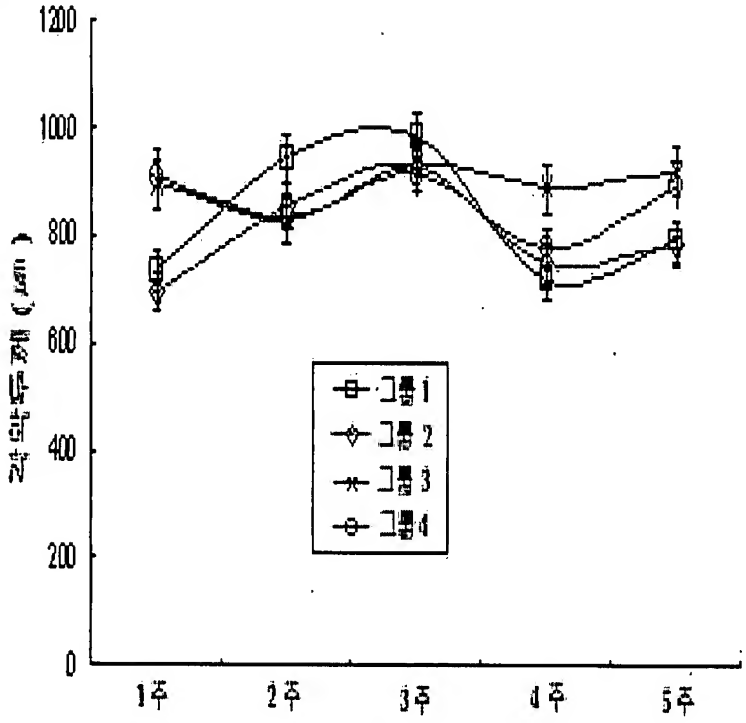
The manufacturing method of the amniotic extract of claim 7, wherein the amniotic extract mixes with the methyl cellulose and it puts into the disease in which an anti-ultraviolet is possible and it refrigerates and keeps at low temperature.

Drawing(s)









Drawings